Diet-Induced Obesity Influences Oocyte Developmental Competence Via Peroxisome Proliferator-Activated Receptor Gamma (PPARG)-Mediated Mechanisms

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A thesis submitted for the degree of Doctor of Philosophy

November 2008

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Declaration

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Cadence E Minge

November 2008

Acknowledgements

I would thank my two supervisors, Prof. Robert Norman and Dr. Rebecca Robker who provided me with the opportunity to be involved in their research. Both Rob and Becky provided unwavering support and continuous encouragement throughout my PhD studies, and I will forever be thankful.

In addition I would like to thank Prof. Marc-André Sirard and Isabel Dufort at the Centre de Recherche en Biologie de la Reproduction, Departement des Sciences Animales, at Universite Laval, Quebec, Canada who generously allowed me to utilise their group's established microarray facilities, and who were the most gracious of hosts.

I would also like to acknowledge the members Discipline of Obstetrics and Gynaecology at the University of Adelaide who assisted me during my studies, particularly Prof. Julie Owens (from the Early Life Programming of Health and Disease group), Dr. Michelle Lane (and the other members of the Oocyte and Early Embryo Development group) and A/Prof. David Kennaway (from the Circadian Physiology Group) for their expert advice and tutorage. I would also like to acknowledge Dr. Victoria Tsargareli and Lisa Akison for their extensive hands-on help throughout this study. My most sincere thanks are extended to Brenton Bennett, to whom I am forever indebted. Never were mice that needed tagging, or embryos that needed moving, left wanting. Thanks also to all the O&G PhD students who provided celebrations for the successes, and support for the most challenging moments of my candidature.

These studies were carried out using financial support obtained from grants from the National Health and Medical Research Council and University of Adelaide. I would like to acknowledge the Faculty of Health Science, Research Centre for Reproductive Health and Discipline of Obstetrics and Gynaecology for providing my postgraduate scholarship and travel grants.

Also, thank you to my family: Mum and Dad, Denham and Mardi, Grandma and Bob, Phil and Vicki, Thomas and Claire. You all made sure I was sustained with excellent meals, exercised with engaging discussions, and nourished by unending love. At home, thank you to Harvey, who helped with all tasks pertaining to belly rubs and pats, and offered consistent reassurance that there would always be a warm and furry friend waiting up for me when I got home late.

And most of all, thank you to my husband Nick. Such kindness and patience as is always given freely by you, made this hard work feel easy. And at last my love, it is done.

Abstract

Across the world more women of childbearing age are becoming overweight and obese. Although overweight women have similar co-morbidity and stigmata as men they also experience problems specific to their gender. In particular, there is significant evidence that overweight and obese women require a longer time to successfully conceive, suggesting influence of bodyweight and adipose tissue mass upon the events surrounding conception.

This thesis investigated the interaction between diet-induced obesity and female reproductive function. To achieve this, the influence of maternal obesity-induced insulin resistance on ovulation and oocyte health, as indicated by subsequent embryonic developmental competence was determined.

Obesity adversely affects many aspects of health, and rodent models of diet-induced obesity are commonly used to investigate these consequences. However the impact of strain and genetic background on phenotypic response to diet, particularly in females, has not been systematically defined. We therefore characterised female metabolic responses of five different strains of laboratory mouse (Swiss, Balb/c, C57BL/6, CBA/CaH and 129T2Sv/Ems) to a “Western” high fat diet (22% fat, 0.15% cholesterol) and matched control diet (6% fat, 0% cholesterol). After 16 weeks of diet exposure the development and extent of hyperglycaemia, hyperinsulinaemia, insulin resistance, dyslipidaemia, and markers of chronically inflamed adipose tissue depots varied profoundly across the different strains.

To then determine if a perturbed metabolic profile triggers female infertility, these female mice were mated with strain matched, non-obese males, and zygotes extracted from the reproductive tract immediately following fertilization. Despite strain-dependent variation in susceptibility to the development of obesity, dyslipidaemia and insulin resistance, all mice investigated exhibit some degree of impaired reproductive potential following exposure to a high fat diet. We documented alteration to ovulation incidence and rate, fertilization, early embryo development to the blastocyst stage, and blastomere differentiation into the inner cell mass and trophectoderm cell lineages.

The nature of obesity-induced perturbation of female reproductive processes was more closely examined using statistical modelling which identified the specific metabolic parameters that were strongly associated with reproductive defects. These associations were consistent across the range of genetic backgrounds assessed and highlighted key mediators of this interaction, in particular, insulin resistance.

To determine if ovarian gene products already implicated in other reproductive outcomes are differentially regulated under conditions of obesity, ovarian mRNA collected at the pro-estrous (pre-ovulatory) stage of the reproductive cycle was applied to microarray slides developed through Suppressive Subtractive Hybridization. Two different gene chips that were enriched for ovarian genes were used. A number of genes were minimally regulated, and there was lack of significant validation in subsequent, and larger, sample cohorts. These findings have provided substantial technical information, and new experimental designs that overcome the current limitations have been established to obtain more informative data.

The role that insulin resistance plays in folliculogenesis and the development of oocyte developmental competence was more closely investigated. Hyperinsulinemia can interfere directly with ovarian cell function or be indirectly associated with other hormonal conditions detrimental to optimal fertility. To reverse the effects of obesity/hyperinsulinemia and identify the signalling pathways responsible for disruption of pre-implantation events, obese female mice were treated for 4 days prior to mating with three different insulin-sensitizing and plasma glucose-reducing pharmaceuticals: glucose and lipid-lowering AMP Kinase activator, AICAR, 30mg/kg/day; IκK inhibitor that reverses insulin resistance, sodium salicylate, 50mg/kg/day; or Peroxisome Proliferator-Activated Receptor Gamma (PPARG) agonist rosiglitazone, 10mg/kg/day. AICAR or sodium salicylate treatment did not have significant effects on the reproductive parameters examined. However, embryonic development to the blastocyst stage was significantly improved when diet-induced obese mice were treated with rosiglitazone, effectively repairing development rates. Rosiglitazone also normalized obesity-associated abnormal blastomere allocation to the inner cell mass. Such improvements to oocyte quality were coupled with weight loss, improved glucose metabolism and changes in ovarian mRNA expression of PPARG-regulated cholesterol transporters.

Overall, this thesis has demonstrated for the first time a link between maternal obesity and the ovarian follicle can impede oocyte health and developmental potential. As a result, the oocyte released at ovulation expresses impaired developmental competence following to conception. Key cellular pathways have been identified in this relationship, specifically PPARG-directed cell responses.

Publications arising from this thesis:

- **Minge CE, Bennett BD, Norman RJ, Robker RL** *Peroxisome Proliferator-Activated Receptor gamma Agonist Rosiglitazone Reverses the Adverse Effects of Diet-Induced Obesity on Oocyte Quality.* Endocrinology 2008,**149**:2646-2656

- **Minge CE, Robker RL, Norman RJ** *PPAR Gamma: Coordinating Metabolic and Immune Contributions to Female Fertility.* PPAR Research 2008:243791 (in press).

- **Minge CE, Bennett BD, Tsagareli V, Lane M, Owens JA, Norman RJ, Robker RL** *Inflammatory and Metabolic Syndrome Phenotypes are Strain-Dependent in Female Mice with Diet-Induced Obesity* (in preparation)

- **Minge CE, Bennett BD, Tsagareli V, Davies MJ, Owens JA, Norman RJ, Robker RL** *Strain-specific Adverse Effects of a High Fat Diet on Ovulation and Oocyte Quality in the Mouse* (in preparation)

Abstracts arising:

2007

- **Minge CE** *Peroxisome Proliferator-Activated Receptor gamma (PPARγ) Agonist Rosiglitazone Reverses the Adverse Effects of Diet-Induced Obesity on Ovarian Function and Female Fertility.* Young Investigator of the Year, October 2007, Adelaide, Australia. (The Young Investigator Award is a highly successful event rewarding excellence in South Australia's young researchers in both science and their ability to communicate and 'sell' that science.)

- **Minge CE, Bennett BD, Lane M, Norman RJ, Robker RL** *Impaired oocyte developmental competence arises from diet-induced obesity and can be reversed by peri-ovulatory rosiglitazone treatment.* Annual Meeting of the Society for Reproductive Biology, September 2007, Christchurch, New Zealand.

- **Minge CE, Bennett BD, Lane M, Norman RJ, Robker RL** *Obesity-Induced Female Infertility Arises From Impaired Oocyte Developmental Competence And Can Be Reversed By Peri-*

Ovulatory Rosiglitazone Treatment. Ross Wishart Memorial Award, SA Australian Society for Medical Research (ASMR) Scientific Meeting, June 2007, Adelaide, Australia. (The Ross Wishart Memorial Award is presented to the most outstanding postgraduate presentation at the South Australian ASMR Annual Scientific Meeting.)

2006

- **Minge CE, Bennett BD, Lane M, Norman RJ, Robker RL** *Obesity-Induced Female Infertility Arises From Impaired Oocyte Developmental Competence And Can Be Reversed By Peri-Ovulatory Rosiglitazone Treatment.* 10th International Congress on Obesity, September 2006, Sydney, Australia.

- **Minge CE** PPARγ *Agonist Rosiglitazone Reverses the Adverse Effects of Diet-Induced Obesity on Ovarian Function and Female Fertility.* Young Investigator Award Semi-Finals, September 2006, Adelaide, Australia.

- **Minge CE, Bennett BD, Lane M, Norman RJ, Robker RL** *Peri-Ovulatory Rosiglitazone Treatment Reverses Obesity-Induced Female Infertility Arising From Impaired Oocyte Developmental Competence.* 39th Annual Meeting of the Society for the Study of Reproduction, August 2006, Omaha, USA.

- **Minge CE, Bennett BD, Tsagareli V, Norman RJ, Lane M, Robker RL**. *Ovulation and Oocyte Quality are Reduced in Mice with Diet-Induced Obesity.* 88th Annual Meeting of the Endocrine Society, June 2006, Boston, USA.

- **Minge CE, Bennett BD, Tsagareli V, Norman RJ, Lane M, Robker RL** *Ovulation and Oocyte Quality are Reduced in Mice with Diet-Induced Obesity.* "Five minutes of excitement and fame", "Womb to Tomb" Human Reproductive Health Throughout the Ages International Congress, March 2006, Adelaide Australia.

2005

- **Minge CE, Bennett BD, Tsagareli V, Norman RJ, Lane M, Robker RL** *Effects of Diet-Induced Obesity on Ovarian Function and Female Fertility*. Annual Meeting of the Society for Reproductive Biology, September 2005, Perth, Australia.

Abbreviations

17β-HSD	17 beta-hydroxysteroid dehydrogenase
3β-HSD	3 beta-hydroxysteroid dehydrogenase
A	androgen
aaRNA	uridine 5'-triphosphate-amino allyl ribonucleic acid
AcLDL	acetylated low density lipoprotein
AGE	advanced glycated ends
AGER	advanced glycated ends receptor
AICAR	5-aminoimidazole 4-carboxamide-riboside
AMPK	5'AMP-activated protein kinase
ANOVA	analysis of variance
ART	assisted reproductive technologies
AUC	area under the curve
BMI	body mass index
BMPs	bone morphogenic proteins
CC	clomiphene citrate
CD	control diet
cDNA	complementary DNA
CE	cholesterol ester
CETP	cholesteryl ester transfer protein
CL	corpus luteum
cm	centimetres
COC	cumulus oocyte complex
CRP	C-reactive protein
CT	threshold cycle
CYP17	cytochrome P450cyp17
DHEA	dehydroepiandrosterone
DHEA-S	dehydroepiandrosterone-sulfate
DIO	diet-induced obesity
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid

E/E2	estrogen
EDTA	ethylenediaminetetraacetic acid
FAI	free androgen index
FF	follicular fluid
FFA	free fatty acids
FSH	follicle stimulating hormone
g	grams
GDF9	growth differentiation factor 9
GEE	generalized estimating equation
GnRH	gonadotrophin releasing hormone
GV	germinal vesicle
GVBD	germinal vesicle breakdown
h	hours
HbA(1C)	hemaglobin A1C (glycosylated hemaglobin)
HDL	high density lipoprotein
HDL-C	HDL-cholesterol
HFD	high fat diet
HOMA	homeostasis model of assessment
HOMA-IR	HOMA-insulin resistance
ICM	inner cell mass
ICSI	intra-cytoplasmic sperm injection
IGF	insulin-like growth factor
IGFBP	IGF binding protein
IKK-b	IκB kinase beta
IL	interleukin
IPGTT	intraperitoneal glucose tolerance test
IU	international units
IVF	in vitro fertilzation
kg	kilograms
L	litres
LDL	low density lipoprotein
LDL-C	LDL-cholesterol

LH	luteinizing hormone
LMD	laser microdissection
M	metres
mg	milligrams
MJ	mega joules
ml	millilitres
mmol	milli mole
mRNA	messenger RNA
MZT	maternal-zygotic transition
NF-κB	nuclear factor-kappa B
ng	nanograms
NSAID	non-steroidal anti-inflammatory drugs
OGTT	oral glucose tolerance test
oxLDL	oxidized LDL
PBR	peripheral benzodiazepine receptor
PCOS	polycystic ovary syndrome
PCR	polymerase chain reaction
pg	picograms
pmol	pico mole
PPARG	peroxisome proliferator activated receptor–gamma
PPRE	PPAR response element
QUICKI	quantitative insulin-sensitivity check index
R2	coefficient of determination
RIA	radio-immuno assay
RNA	ribonucleic acid
ROS	reactive oxygen species
RT	reverse transcription
RXR	retinoid X receptor
SDS	sodium dodecyl sulfate
SEM	standard error
SHBG	sex hormone binding globulin
SSH	Suppression subtractive hybridization

StAR	steroidogenic acute regulatory protein
T	testosterone
TE	trophectoderm
TGFβ	Transforming growth factor beta
TNFα	Tumor necrosis factor alpha
UCP	uncoupling protein
μg	micrograms
μl	microlitres
μm	micrometres
Vol	volume
WHO	World Health Organization
WHR	waist-hip ratio
Wt	weight

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